Desarrollo de vacunas orales basadas en proteinas recombinantes derivadas de la toxina del colera.
Sanchez J; Solorzano R M
Centro de Investigaciones sobre Enfermedades Infecciosas, Instituto Nacional de Salud Publica, Cuernavaca, Morelos, Mexico.
Salud p'ublica de M'exico (MEXICO) May-Jun 1992, 34 (3) p287-91, ISSN 0036-3634 Journal Code: 0404371
Document type: Journal Article ; English Abstract
Languages: SPANISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
In this paper a new approach to create antigens through genetic engineering is discussed. In this particular case the subunits of V. cholerae toxin are used as heterologous epitope carries. In this paper the manipulation of A and B subunits is described. This manipulation allows both the **insertion** of epitopes to the B subunit and the use of subunit A in the construction of recombinant antigens similar to the ones derived from subunit B.
Descriptors: *Bacterial Vaccines; *Cholera Toxin; *Genetic Engineering; *Recombinant Proteins; Administration, Oral; Bacterial Vaccines --administration and dosage--AD; **Cholera Toxin** --genetics--GE; Epitopes; Vibrio cholerae--genetics--GE
CAS Registry No.: 0 (Bacterial Vaccines); 0 (Epitopes); 0 (Recombinant Proteins); 9012-63-9 (Cholera Toxin)
Record Date Created: 19920729
Record Date Completed: 19920729

27/9/44 (Item 44 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09348437 PMID: 1377408
[Development of oral vaccines based on recombinant proteins derived from cholera toxin]
[illegible]
Noda M
Nippon saikingaku zasshi. Japanese journal of bacteriology (JAPAN) Mar 1992, 47 (2) p367-72, ISSN 0021-4930 Journal Code: 2984804R
Document type: Journal Article; Review; Review, Tutorial
Languages: JAPANESE
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
(15 Refs.)
Tags: Human
Descriptors: Adenylate Cyclase--metabolism--ME; *Cholera--etiology--ET; *Cholera Toxin --toxicity--TO; *GTP-Binding Proteins--physiology--PH; ADP-Ribosylation Factors; Adenosine Diphosphate--metabolism--ME; Cholera --metabolism--ME; Cholera Toxin--metabolism--ME; GTP-Binding Proteins --metabolism--ME; Guanosine Triphosphate--metabolism--ME; Protein Binding
CAS Registry No.: 58-64-0 (Adenosine Diphosphate); 86-01-1 (Guanosine Triphosphate); 9012-63-9 (Cholera Toxin)
Enzyme No.: EC 3.6.1.- (GTP-Binding Proteins); EC 3.6.1. **47** (ADP-Ribosylation Factors); EC 4.6.1.1 (Adenylate Cyclase)
Record Date Created: 19920720
Record Date Completed: 19920720

27/9/51 (Item 51 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08540053 PMID: 2344953
[Inheritance and expression of cholera toxin genes introduced into Vibrio cholerae el tor cells in a hybrid plasmid]
Osobennosti nasledovaniia i ekspressii genov kholernogo toksina,

vvedennykh v kletki Vibrio cholerae eltor v sostave gibridnoi plazmidy.
Smirnova N I; Eroshenko G A; Livanova L F; Mozharov O T; Fil'kova S L; Il'ina T S
Genetika (USSR) Feb 1990, 26 (2) p206-14, ISSN 0016-6758 Journal Code: 0047354
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Two replicons, pOX38 (in F-factor derivative lacking all IS elements) and pCT105 (containing cholera toxin operon cloned in pBR322) have been fused to produce recombinant plasmid, pCO109, which was then introduced into Vibrio cholerae eltor by conjugation. Restriction analysis showed pCO109 to dissociate in V. cholerae cells at a higher frequency than in Escherichia coli strains, its pOX38 component being lost, while the pCT105 component demonstrated relative stability. V. cholerae eltor RV79 (pCT105) produced 4-5 micrograms/ml of cholera toxin. Occasional **insertion** of cloned vctA, B operon into RV79 chromosome was also observed.

Descriptors: **Cholera Toxin** --genetics--GE; *Gene Expression Regulation, Bacterial; *Genes, Bacterial; *Plasmids; *Vibrio cholerae--genetics--GE; Cholera Toxin--biosynthesis--BI; Conjugation, Genetic; Recombination, Genetic; Vibrio cholerae--metabolism--ME
CAS Registry No.: 0 (Plasmids); 9012-63-9 (Cholera Toxin)
Record Date Created: 19900703
Record Date Completed: 19900703

27/9/60 (Item 60 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07050257 PMID: 3949714

Effect of a recA mutation on cholera toxin gene amplification and deletion events.

Goldberg I; Mekalanos J J
Journal of bacteriology (UNITED STATES) Mar 1986, 165 (3) p723-31, ISSN 0021-9193 Journal Code: 2985120R
Contract/Grant No.: AI-18045; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The cholera toxin operon (ctxAB) is located on a 7-kilobase pair variable genetic element which undergoes genetic duplication and amplification events in Vibrio cholerae. Amplification of the ctx genetic element was investigated by substituting the resident ctx loci of two V. cholerae strains with a DNA fragment encoding resistance to kanamycin. Although these strains were not normally resistant to greater than 150 micrograms of kanamycin per ml, spontaneous derivatives could be obtained that grew well on 3 mg of kanamycin per ml. Southern blot analysis of these highly resistant isolates demonstrated that the ctx element was amplified approximately 20-fold. This amplification process was completely inhibited in the absence of a functional recA gene. The V. cholerae RecA protein, therefore, is essential for cholera toxin gene amplification. Spontaneous **deletions** of the ctx structural genes were observed in both recA+ and recA- V. cholerae strains, although such **deletions** occurred at a 21-fold-lower frequency in the latter case. Structural analysis of these ctx amplification and **deletion** events supports a model for their formation that involves unequal crossing over between repetitive sequences located upstream and downstream of the ctx operon.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: **Cholera Toxin** --genetics--GE; *Chromosome **Deletion** ; *Gene Amplification; *Rec A Recombinases--physiology--PH; *Recombination, Genetic ; *Vibrio cholerae--genetics--GE; Crossing Over (Genetics); Drug Resistance, Microbial; Genes, Bacterial; Genes, Structural; Kanamycin

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Set     Items   Description
S1          9   CTX? (2N) CRM?
S2          1   'CTX ANALOGS'
S3        602   'CHOLERA TOXIN --GENETICS --GE' OR 'CHOLERA TOXIN --INHALA-
              TIONAL DRUG ADMINISTRATI' OR 'CHOLERA TOXIN --INTRACEREBROVEN-
              TRICULAR DRUG A' OR 'CHOLERA TOXIN --INTRADERMAL DRUG ADMINIS-
              TRATIO'
S4        139   E37-E44 OR E47 OR E48
S5         68   'CHOLERA TOXIN --PHARMACEUTICS --PR'
S6        379   'CHOLERA TOXIN --TOXICITY --TO'
S7         42   'CHOLERA TOXIN --THERAPEUTIC USE --TU'
S8        379   'CHOLERA TOXIN --TOXICITY --TO'
S9          1   'CHOLERA TOXIN B SUBUNIT CHIMERA'
S10         8   'CHOLERA TOXIN DERIVATIVE'
S11         1   'CHOLERA TOXIN DERIVATIVES'
S12        33   'CHOLERA TOXIN DIPHTHERIA TOXIN CYTOTOXICITY' OR S6P
S13        11   'CHOLERA TOXIN EPIDERMAL GROWTH FACTOR ORAL MUC' OR E16P
S14         1   'CHOLERA TOXIN HEAT LABILE ENTERO TOXIN LYMPHAT'
S15         1   'CHOLERA TOXIN HEAT-LABILE ENTEROTOXIN TYPE I A'
S16         1   'CHOLERA TOXIN MAPPING'
S17        10   'CHOLERA TOXIN MUCOSAL ADJUVANT' OR 'CHOLERA TOXIN MUTANT'
              OR 'CHOLERA TOXIN MUCOSAL IMMUNIZATION'
S18         2   'CHOLERA TOXIN NON TOXIC DERIVATIVE' OR 'CHOLERA TOXIN NON-
              -TOXIC B SUBUNIT'
S19         1   'CHOLERA TOXIN PRODUCTION MUTAGEN SUBUNITS'
S20         1   'CHOLERA TOXIN SUBUNITS'
S21         2   'CHOLERA TOXIN VARIANT' OR 'CHOLERA TOXIN VARIANT A SUBUNI-
              TS'
S22         2   E32-E36
S23      1225   S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 -
              OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17OR S18 OR S19
              OR S20 OR S21
S24       846   RD (unique items)
S25       395   S24/1999:2004
S26       451   S24 NOT S25
S27        67   S26 AND (INSERT? OR DELET? OR 29 OR 47 OR GLU OR GLUTAMIC?
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 27/9/4     (Item 4 from file: 155)
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13952514   PMID: 9651815
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Analysis of mechanisms of epidermal proliferation induced by intracutaneous injection of cholera toxin by the use of site-specifically mutated cholera toxins.

Yamaoka J; Imamura S
Department of Dermatology, Kyoto University Hospital, Japan.

Journal of dermatological science (IRELAND) Mar 1998, 16 (3) p182-90
, ISSN 0923-1811 Journal Code: 9011485
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Intracutaneous injection of cholera toxin (CT) into rabbits increases vascular permeability and induces epidermal proliferation. To understand the mechanisms of these effects on the skin, we evaluated the involvement of the ADP-ribosyltransferase activity of the A subunit of CT and receptor-binding interactions between GM1-ganglioside and the B subunit of CT. We constructed two mutant CTs, E112K and W88K, by site-directed

mutagenesis. Mutant CT-E112K, in which **glutamic** acid at position 112 (E112) of the A subunit of CT was replaced by lysine, has been shown to have lost its biological activity on Chinese hamster ovary (CHO) cells because of its abolished ADP-ribosyltransferase activity. Mutant CT-W88K, in which tryptophan at position 88 (W88) of the B subunit of CT was replaced by lysine, has been shown to have lost its binding ability to GM1-ganglioside. Intracutaneous injection of these mutant CTs evoked less vascular permeability and less epidermal proliferation than recombinant wild-type CT. These results suggest that: (1) the ADP-ribosyltransferase activity carried by E112 of the A subunit of CT; and (2) the binding ability to GM1-ganglioside via W88 of the B subunit of CT are essential for these effects of CT on the skin.

Descriptors: **Cholera Toxin** --genetics--GE; *Cholera Toxin--pharmacology --PD; *Epidermis--cytology--CY; *Epidermis--drug effects--DE; Animals; CHO Cells; Capillary Permeability--drug effects--DE; Cell Division --drug effects--DE; Chemistry, Physical; Cholera Toxin--chemistry--CH; Hamsters; Injections, Intradermal; Mutagenesis, Site-Directed; Mutation--physiology --PH; Rabbits; Recombinant Proteins

CAS Registry No.: 0 (Recombinant Proteins); 9012-63-9 (Cholera Toxin)

Record Date Created: 19980918

Record Date Completed: 19980918

27/9/8 (Item 8 from file: 155)

13431257 PMID: 9104807

Mutants in the ADP-ribosyltransferase cleft of cholera toxin lack diarrheagenicity but retain adjuvanticity.

Yamamoto S; Takeda Y; Yamamoto M; Kurazono H; Imaoka K; Yamamoto M; Fujihashi K; Noda M; Kiyono H; McGhee J R

Immunobiology Vaccine Center and Department of Microbiology, University of Alabama at Birmingham, 35294, USA.

Journal of experimental medicine (UNITED STATES) Apr 7 1997, 185 (7) p1203-10, ISSN 0022-1007 Journal Code: 2985109R

Contract/Grant No.: AI 18958; AI; NIAID; DE 04217; DE; NIDCR; DK 44240; DK; NIDDK; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS; AIDS/HIV

Cholera toxin (CT), the most commonly used mucosal adjuvant in experimental animals, is unsuitable for humans because of potent diarrhea-inducing properties. We have constructed two CT-A subunit mutants, e.g., serine-->phenylalanine at position 61 (S61F), and **glutamic** acid-->lysine at 112 (E112K) by site-directed mutagenesis. Neither mutant CT (mCT), in contrast to native CT (nCT), induced adenosine diphosphate-ribosylation, cyclic adenosine monophosphate formation, or fluid accumulation in ligated mouse ileal loops. Both mCTs retained adjuvant properties, since mice given ovalbumin (OVA) subcutaneously with mCTs or nCT, but not OVA alone developed high-titered serum anti-OVA immunoglobulin G (IgG) antibodies (Abs) which were largely of IgG1 and IgG2b subclasses. Although nCT induced brisk IgE Ab responses, both mCTs elicited lower anti-OVA IgE Abs. OVA-specific CD4+ T cells were induced by nCT and by mCTs, and quantitative analysis of secreted cytokines and mRNA revealed a T helper cell 2 (Th2)-type response. These results now show that the toxic properties of CT can be separated from adjuvanticity, and the mCTs induce Ab responses via a Th2 cell pathway.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: Adjuvants, Immunologic--toxicity--TO; * **Cholera Toxin** --toxicity--TO; *Diarrhea; *Mutation; *Poly(ADP-ribose) Polymerases --genetics--GE; Animals; CD4-Positive T-Lymphocytes--immunology--IM; CHO Cells; **Cholera Toxin** --genetics--GE; Cyclic AMP; Cytokines--biosynthesis --BI; Dose-Response Relationship, Drug; Hamsters; Ileum--drug effects--DE; Lymphocyte Activation; Mice; Mice, Inbred C57BL; Mucous Membrane

--immunology--IM; Th2 Cells--immunology--IM; Toxicity Tests
CAS Registry No.: 0 (Adjuvants, Immunologic); 0 (Cytokines); 60-92-4 (Cyclic AMP); 9012-63-9 (Cholera Toxin)
Enzyme No.: EC 2.4.2.30 (Poly(ADP-ribose) Polymerases)
Record Date Created: 19970514
Record Date Completed: 19970514

27/9/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13184535 PMID: 8854382
Genetic manipulation of Vibrio cholerae for vaccine development: construction of live attenuated El Tor candidate vaccine strains.
Benitez J A; Silva A J; Rodriguez B L; Fando R; Campos J; Robert A; Garcia H; Garcia L; Perez J L; Oliva R; Torres C A; Ledon T
Grupo de Genetico del Centrol Nacional de Investigaciones Cientificas, Havana, Cuba.
Archives of medical research (MEXICO) Autumn 1996, 27 (3) p275-83, ISSN 0188-4409 Journal Code: 9312706
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The recent spread of El Tor cholera to America augments the need for an effective, safe and economical vaccine. In the present paper we describe the construction of live attenuated V. Cholerae strains by specifically **deleting** the genes encoding cholera toxin and other putative toxins from the bacterial chromosome. To maximize the likelihood of exposing protective antigens relevant to currently circulating vibrios we selected for genetic manipulation recent epidemic V. cholerae isolates from Peru. The mutant strains did not produce cholera toxin in vitro and in vivo. **Deletion** of the virulence cassette was accompanied by marked attenuation in the infant mouse cholera model. A selected El Tor Ogawa candidate vaccine strain was refractory to acquisition of foreign genes by conjugation with toxigenic vibrios.
Descriptors: **Cholera Toxin** --genetics--GE; *Cholera Vaccines; *Gene **Deletion** ; *Vaccines, Attenuated; *Vibrio cholerae--genetics--GE; Animals; Antigens, Bacterial--immunology--IM; Chromosomes, Bacterial--genetics--GE; Conjugation, Genetic; Genes, Structural, Bacterial; Mice; Rabbits; Vibrio cholerae--classification--CL; Vibrio cholerae--immunology--IM; Vibrio cholerae--pathogenicity--PY; Virulence--genetics--GE
CAS Registry No.: 0 (Antigens, Bacterial); 0 (Cholera Vaccines); 0 (Vaccines, Attenuated); 9012-63-9 (Cholera Toxin)
Record Date Created: 19961226
Record Date Completed: 19961226

27/9/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13117358 PMID: 8783505
Mechanism of action of cholera toxin & other toxins.
Ganguly N K; Kaur T
Department of Experimental Medicine & Biotechnology, Postgraduate Institute of Medical Education & Research, Chandigarh.
Indian journal of medical research (INDIA) Jul 1996, 104 p28-37, ISSN 0971-5916 Journal Code: 0374701
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
Vibrio cholerae produce a variety of extracellular products that have

deleterious effects on eukaryotic cells. The massive diarrhoea produced by V. cholerae is caused by cholera toxin (CT). CT is composed of 1A and 5B units. CT causes a significant amount of fluid secretion and haemorrhage in the ligated rabbit ileal loops. Its action involves the role of various biochemical pathways. CT acts by activation of adenylate cyclase-cAMP system located at the basolateral membrane of intestinal epithelial cells. The increase in cyclic AMP levels is mainly responsible for the altered transport of Na+ and Cl-. Besides activating cAMP, CT is also known to act through release of prostaglandins and involvement of intramural nerves. Besides CT, other bacterial toxins like Escherichia coli LT, Salmonella toxin, Shigella toxin and Campylobacter toxin also possess A-B structure. The structure and function of E. coli LT resembles closely that of CT. Most of the bacterial toxins exert their effect through involvement of ADP-ribosylating proteins whereas other toxins involve guanylate cyclase system, calcium and protein kinases for their ultimate action. (84 Refs.)

Tags: Human

Descriptors: **Cholera Toxin** --toxicity--TO; Adenylate Cyclase--metabolism --ME; Animals; Bacterial Toxins--toxicity--TO; Cholera Toxin--chemistry--CH ; Enterotoxins--toxicity--TO; Prostaglandins--physiology--PH; Rabbits; Shiga Toxins

CAS Registry No.: 0 (Bacterial Toxins); 0 (Enterotoxins); 0 (Prostaglandins); 0 (Shiga Toxins); 0 (enterotoxin LT); 0 (heat stable toxin (E coli)); 9012-63-9 (Cholera Toxin)

Enzyme No.: EC 4.6.1.1 (Adenylate Cyclase)

Record Date Created: 19961017

Record Date Completed: 19961017

27/9/21 (Item 21 from file: 155)

12787871 PMID: 7551060

Cholera toxin (CTX) genetic element in Vibrio cholerae O139.

Bhadra R K; Roychoudhury S; Banerjee R K; Kar S; Majumdar R; Sengupta S; Chatterjee S; Khetawat G; Das J

Biophysics Division, Indian Institute of Chemical Biology, Calcutta.

Microbiology (Reading, England) (ENGLAND) Aug 1995, 141 (Pt 8) p1977-83, ISSN 1350-0872 Journal Code: 9430468

Erratum in Microbiology 1995 Dec;141(Pt 12) 3251

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

PFGE analysis of the NotI- and SfiI-digested genome of Vibrio cholerae O139 strains isolated from different epidemic regions of India showed that all the strains are of clonal origin and the genome size is about 2.2 Mb. An analysis of the electrophoretic profiles of the genome of O139 strains, the RFLP of the cholera toxin (ctx) gene and Southern blot hybridization of NotI-digested genomes of classical, El Tor and O139 with a NotI-linking clone of classical strain 569B, suggest that these strains closely resemble V. cholerae O1 biotype El Tor, but are widely different from the classical O1 vibrios. Using restriction enzymes which cleave a single site in either the core region or in the direct repeat sequence (RS) of the CTX genetic element, it has been shown that the genome of most of the O139 strains has two copies of the ctx gene in tandem connected by two RSs. The chromosomal location of the CTX genetic element in the O139 strain is the same as that reported for El Tor vibrios. The organization of the virulence gene cassettes in different O139 strains shows genetic heterogeneity in the population. Whilst most of the epidemic strains have two copies of the CTX genetic element, in some strains the number of elements has been amplified and in at least one strain a single copy of the element has been **deleted** .

Tags: Support, Non-U.S. Gov't

Descriptors: **Cholera Toxin** --genetics--GE; *DNA, Bacterial--analysis--AN ; *Gene Dosage; *Genome, Bacterial; *Vibrio cholerae--genetics--GE;

Blotting, Southern; Deoxyribonucleases, Type II Site-Specific; Electrophoresis, Agar Gel; Electrophoresis, Gel, Pulsed-Field; Polymorphism, Restriction Fragment Length; Repetitive Sequences, Nucleic Acid; Restriction Mapping
CAS Registry No.: 0 (DNA, Bacterial); 9012-63-9 (Cholera Toxin)
Enzyme No.: EC 3.1.21.- (endodeoxyribonuclease NotI); EC 3.1.21.- (endodeoxyribonuclease SfiI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific)
Gene Symbol: ace; cep; ctxAB; zot
Record Date Created: 19951106
Record Date Completed: 19951106

27/9/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12541059 PMID: 7872438
Molecular characterization of Vibrio cholerae O139 isolates from Asia.
Echeverria P; Hoge C W; Bodhidatta L; Serichantalergs O; Dalsgaard A; Eampokalap B; Perrault J; Pazzaglia G; O'Hanley P; English C
Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.
American journal of tropical medicine and hygiene (UNITED STATES) Feb 1995, 52 (2) p124-7, ISSN 0002-9637 Journal Code: 0370507
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: AIM; INDEX MEDICUS
In 1992, a serologically novel clone of Vibrio cholerae, designated O139, caused large epidemics of diarrhea in India and Bangladesh. To determine the extent of the spread of V. cholerae O139 worldwide, 484 V. cholerae non-O1 strains isolated from different patients with diarrhea in Thailand, Indonesia, the Philippines, and Peru in 1993 were tested for agglutination in O139 antisera. One hundred fifty-one of these 484 isolates were examined for genes encoding cholera toxin, zonula occlulans toxin, the repetitive sequence 1, and the toxin coregulated pilin A (the V. cholerae virulence gene complex). Thirty-three percent (122 of 364) of V. cholerae non-O1 strains isolated from different patients with diarrhea in Thailand agglutinated in O139 antisera. Ninety-eight percent (120 of 122) of V. cholerae O139 contained the V. cholerae virulence gene complex. None of the 104 V. cholerae non-O1 strains isolated from patients with diarrhea in Indonesia or the 14 strains from patients with diarrhea in the Philippines were serotype O139. Four different ribotypes were found in V. cholerae O139 isolated in Asia. Twenty-three (**47** %) of 49 Thai O139 strains examined were of different ribotypes than isolates from India and Bangladesh; V. cholerae strains that were not O1 or O139 that were isolated from flies and water in Thailand 11 years previously in 1981 contained the same V. cholerae virulence gene complex found in V. cholerae O1 and O139. This suggests that other unidentified virulence determinants are involved in V. cholerae O139 pathogenesis.
Tags: Comparative Study; Female; Human; Support, U.S. Gov't, Non-P.H.S.
Descriptors: *Cholera--microbiology--MI; *DNA, Bacterial--analysis--AN; *Diarrhea--microbiology--MI; *Vibrio cholerae--genetics--GE; Cholera --epidemiology--EP; **Cholera Toxin** --genetics--GE; Diarrhea--epidemiology --EP; Disease Outbreaks; Indonesia--epidemiology--EP; Nucleic Acid Hybridization; Peru--epidemiology--EP; Philippines--epidemiology--EP; Repetitive Sequences, Nucleic Acid; Serotyping; Thailand--epidemiology--EP; Vibrio cholerae--classification--CL; Vibrio cholerae--pathogenicity--PY; Virulence--genetics--GE
CAS Registry No.: 0 (DNA, Bacterial); 146481-28-9 (Zot protein); 9012-63-9 (Cholera Toxin)
Record Date Created: 19950330
Record Date Completed: 19950330

27/9/26 (Item 26 from file: 155)

for the wild-type copy in the previously cloned ctxAB operon from El Tor biotype, Ogawa serotype Vibrio cholerae strain 3083, which produces CT-2. Further, the zonula occludens toxin gene, zot, was inactivated by an **insertional** mutation to create the new plasmid construct pCT-2*. Additionally, a DNA fragment encoding the B subunit of CT-1 (CT produced by classical biotype, Inaba serotype V. cholerae strain 569B) was exchanged for the homologous part in pCT-2*, resulting in the creation of pCT-1*. These plasmid constructs were introduced into the CT-negative V. cholerae mutant strain JBK70 (El Tor biotype, Inaba serotype); CT-A-B+ derivatives CVD101 and CVD103 of classical biotype Ogawa and Inaba serotype strains 395 and 569B, respectively; El Tor biotype Inaba and Ogawa serotype strains C6706 and C7258, respectively, recently isolated in Peru; and O139 (synonym Bengal) strain SG25-1 from the current epidemic in India. Recombinant toxins (CT-1* and CT-2*), partially purified from culture supernatants of transformed JBK70, were shown to be inactive on mouse Y1 adrenal tumor cells and in an in vitro ADP-ribosyltransferase assay. CT-1* and CT-2* reacted with polyclonal and monoclonal antibodies against both A and B subunits of CT. The toxin analogs reacted with antibodies against CT-A and CT-B on cellulose acetate strips and in a GM1 enzyme-linked immunosorbent assay; they reacted appropriately with B-subunit epitype-specific monoclonal antibodies in checkerboard immunoblots, and they formed precipitin bands with GM1-ganglioside in Ouchterlony tests. However, the reactions of the modified proteins with anti-A-subunit monoclonal antibodies were weaker than the reactions with wild-type holotoxins. V, cholerae strains carrying ctxA*, with either ctxB-1 or ctxB-2, and inactivated zot genes were created by homologous recombination. The recombinant strains and the purified toxin analogs were inactive in the infant rabbit animal model.(ABSTRACT TRUNCATED AT 400 WORDS)

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *Cholera Toxin--biosynthesis--BI; *Cholera Vaccines --biosynthesis--BI; *Vaccines, Synthetic--biosynthesis--BI; *Vibrio cholerae--genetics--GE; Animals; Base Sequence; **Cholera Toxin** --genetics --GE; **Cholera Toxin** --toxicity--TO; Genes, Bacterial; Molecular Sequence Data; Plasmids; Rabbits

CAS Registry No.: 0 (Cholera Vaccines); 0 (Plasmids); 0 (Vaccines, Synthetic); 9012-63-9 (Cholera Toxin)

Record Date Created: 19940824

Record Date Completed: 19940824

27/9/29 (Item 29 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

10223977 PMID: 7927673

Importance of ADP-ribosylation in the morphological changes of PC12 cells induced by cholera toxin.

Glineur C; Locht C

Unite d'Oncologie Moleculaire, CNRS URA 1160, Institut Pasteur, Lille, France.

Infection and immunity (UNITED STATES) Oct 1994, 62 (10) p4176-85, ISSN 0019-9567 Journal Code: 0246127

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Cholera toxin (CTX) is composed of two subunits, subunit A, which possesses ADP-ribosyltransferase activity, and subunit B, which is responsible for receptor binding. It has previously been shown that agents that increase cyclic AMP (cAMP) levels in cells induce differentiation of PC12 cells into neurite-like cells. In this report, we show that as little as 100 pg of CTX per ml induces such changes. CTX was found to ADP-ribosylate at least four membrane proteins of PC12 cells in vitro and in vivo and to increase intracellular cAMP levels. We have developed an inducible ctx gene expression system in Vibrio cholerae by using the tac promoter. The culture medium of the CTX-producing bacteria was able to

induce the morphological changes and the ADP-ribosylation of the PC12 cell membrane proteins. We have constructed two CTX-cross-reactive mutant proteins (**CTX - CRM**) by site-directed mutagenesis. The choice of **glutamic** acid **29** as the target amino acid was based on sequence similarities with other bacterial toxins. **CTX - CRM** -E29 delta, in which the **Glu - 29** of the A subunit was **deleted** , showed strongly reduced ADP-ribosyltransferase activity and did not induce significant morphological changes of PC12 cells. In contrast, **CTX - CRM** -E29D, in which the **Glu - 29** was replaced by an aspartic acid, was as active as the wild-type protein. We conclude that the ADP-ribosylation activity of CTX is important for the toxin-induced differentiation of PC12 cells. Pertussis toxin, which had no visible effect on PC12 cell morphology, was also able to ADP-ribosylate a membrane-bound protein(s) in vitro and in vivo. Pertussis toxin alone did not significantly increase cAMP levels in PC12 cells, but it acted synergistically with CTX.

Tags: Support, Non-U.S. Gov't

Descriptors: Adenosine Diphosphate Ribose--metabolism--ME; * **Cholera Toxin** --toxicity--TO; Amino Acid Sequence; Animals; Base Sequence; CHO Cells; Cholera Toxin--biosynthesis--BI; **Cholera Toxin** --genetics--GE; Cyclic AMP--analysis--AN; Forskolin--pharmacology--PD; Genetic Vectors; Hamsters; Molecular Sequence Data; PC12 Cells--drug effects--DE; PC12 Cells--metabolism--ME; Rabbits; Rats; Recombinant Proteins--biosynthesis --BI; Recombinant Proteins--toxicity--TO

CAS Registry No.: 0 (Genetic Vectors); 0 (Recombinant Proteins); 20762-30-5 (Adenosine Diphosphate Ribose); 60-92-4 (Cyclic AMP); 66428-89-5 (Forskolin); 9012-63-9 (Cholera Toxin)

Record Date Created: 19941104

Record Date Completed: 19941104

27/9/33 (Item 33 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

10026613 PMID: 7511546

Functional effects of transgenic expression of cholera toxin in pancreatic beta-cells.

Wogensen L; Ma Y H; Grodsky G M; Robertson R P; Burton F; Sutcliffe J G; Sarvetnick N

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Molecular and cellular endocrinology (IRELAND) Dec 1993, 98 (1) p33-42, ISSN 0303-7207 Journal Code: 7500844

Contract/Grant No.: DK01410; DK; NIDDK; DK41801; DK; NIDDK; HD29764; HD; NICHD; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Investigation of intracellular pathways of stimulus-secretion signaling in vivo is possible by transgenic expression of agents known to influence specific biochemical interactions in the cells. The objective of the present study was to establish an experimental model for analyzing signal transduction mechanisms in pancreatic beta-cells in vivo, by expressing the cholera toxin A1 subunit under control of the insulin promoter, intending a constant activation of the Gs-protein, and thereby constant generation of cAMP. Surprisingly, the transgenic mice demonstrated mild hyperglycemia and hypoinsulinemia in vivo, and diminished glucose-induced insulin release from the in vitro perfused pancreas, whereas the pancreatic insulin content was normal. These observations suggest a deficiency in either the insulin release mechanisms or glucose recognition. Although the translated cholera toxin A1 subunit was biologically active, there was no increase in the islet content of cAMP. We conclude that the observed phenotype in the cholera toxin transgenic mice may be caused by a **deleterious** effect of the transgene itself on beta-cell function, or that counter regulatory mechanisms may compensate for the transgene-induced changes in

intracellular enzymatic pathways.
Tags: Female; Human; Male; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Descriptors: *Cholera Toxin--biosynthesis--BI; *Cyclic AMP--physiology --PH; *Islets of Langerhans--metabolism--ME; *Recombinant Fusion Proteins --biosynthesis--BI; *Signal Transduction; 1-Methyl-3-isobutylxanthine --pharmacology--PD; Animals; Base Sequence; **Cholera Toxin** --genetics--GE; Forskolin--pharmacology--PD; GTP-Binding Proteins--metabolism--ME; Glucose --pharmacology--PD; Hyperglycemia--genetics--GE; Insulin--analysis--AN; Insulin--genetics--GE; Insulin--secretion--SE; Islets of Langerhans --secretion--SE; Mice; Mice, Inbred BALB C; Mice, Transgenic; Molecular Sequence Data; Phenotype; Promoter Regions (Genetics); Recombinant Fusion Proteins--genetics--GE
CAS Registry No.: 0 (Recombinant Fusion Proteins); 11061-68-0 (Insulin); 28822-58-4 (1-Methyl-3-isobutylxanthine); 50-99-7 (Glucose) ; 60-92-4 (Cyclic AMP); 66428-89-5 (Forskolin); 9012-63-9 (Cholera Toxin)
Enzyme No.: EC 3.6.1.- (GTP-Binding Proteins)
Record Date Created: 19940505
Record Date Completed: 19940505

27/9/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09845616 PMID: 8406837
CVD110, an attenuated Vibrio cholerae O1 El Tor live oral vaccine strain.
Michalski J; Galen J E; Fasano A; Kaper J B
Department of Medicine, University of Maryland School of Medicine, Baltimore 21201.
Infection and immunity (UNITED STATES) Oct 1993, 61 (10) p4462-8, ISSN 0019-9567 Journal Code: 0246127
Contract/Grant No.: AI 19716; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS
The recent expansion of the seventh cholera pandemic into South America emphasizes the need for a safe, long-lasting, protective, and nonreactogenic vaccine for this disease. Since the predominant Vibrio cholerae O1 strains in the world today are of the El Tor biotype, a bivalent vaccine containing both classical and El Tor biotypes may be desirable. We have constructed a new oral vaccine candidate, V. cholerae CVD110 El Tor, Ogawa, from which all toxin genes so far identified in V. cholerae have been **deleted** . Three of these genes, those encoding cholera toxin (ctx), zonula occludens toxin (zot), and accessory cholera enterotoxin (ace), are located on a 4.5-kb virulence cassette flanked by repetitive sequences (RS1 elements). Homologous recombination between these RS1 elements resulted in the **deletion** of this virulence cassette to yield V. cholerae CVD109. **Insertion** of genes encoding mercury resistance (mer) and the cholera toxin B subunit (ctxB) into the hemolysin locus (hlyA) produced CVD110. This **insertion** serves three purpose. (i) It genetically tags the vaccine strain so as to distinguish it from wild-type V. cholerae O1. (ii) It produces cholera toxin B subunit in order to elicit antitoxic immunity. (iii) It inactivates the hemolysin gene, rendering the strain nonhemolytic on sheep erythrocyte plates. Supernatants from V. cholerae CVD110 cultures are nonreactogenic when assayed in Ussing chambers.
Tags: Support, U.S. Gov't, P.H.S.
Descriptors: *Bacterial Toxins--genetics--GE; *Bacterial Vaccines --immunology--IM; *Vaccines, Attenuated--immunology--IM; *Vaccines, Synthetic--immunology--IM; *Vibrio cholerae--immunology--IM; **Cholera Toxin** --genetics--GE; Genes, Structural, Bacterial; Hemolysins--genetics--GE; Sequence **Deletion**
CAS Registry No.: 0 (Bacterial Toxins); 0 (Bacterial Vaccines); 0 (Hemolysins); 0 (Vaccines, Attenuated); 0 (Vaccines, Synthetic);